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THE REPRODUCTIVE DYSFUNCTION EFFECTS OF GASOLINE INHALATION IN ALBINO RATS.

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Summary: Daily exposure to fuel vapour may pose significant health risk to exposed individuals. Fifteen each of male and female albino rats weighing between 110-230g were divided into test (10) and control (5) groups each. The test animals were exposed to inhalation gasoline for one hour daily for twenty-one consecutive days. All animals were then bled and the serum levels of the reproductive hormones determined. The results showed significant ($P<0.05$) reduction in the serum levels of all the hormones of reproduction in both the male and female test animals. The results suggest that inhalation gasoline exposure significantly ($P<0.05$) lowers the levels of reproductive hormones in albino rats and may thus interfere with reproduction.

Key Words: *Gasoline, Wistar, hormones, reproduction.*

Introduction

Endocrine disrupting compounds come from different sources. Gasoline, popularly known as fuel, is a volatile, highly flammable liquid mixture of hydrocarbons. It is used in internal combustion engines. Gasoline gains entrance into the body orally (accidental), transdermally, and by inhalation. The inhalation route would appear to be more important because more persons may be affected and most exposed persons in Nigeria for instance are unprotected. Gasoline is a mixture of over five hundred hydrocarbons with varied composition according to its place of origin. In general, it contains alkanes, alkenes, alcohol, ether and many additives such as benzene and lead (Isabelle *et al*, 1997). Toxic effects of gasoline and additives range from induction of cancer to disruption of haematopoiesis and endocrine functions (Schlosser *et al*, 1993 and Orzechowski *et al*, 1995). The exposure levels of ethanol (an additive of gasoline) in humans are thought to be lower than levels known to cause toxic effects (Lovei, 1999). In developing countries such as Nigeria where oil exploration is massive, occupational exposure may pose some amount of health risks. Some specific endocrine endpoints are predictive of conceptive female sexual cycle and male fertility status and can be used as early markers of possible reproductive dysfunctions of low dose exposures to gasoline vapour. It is difficult to fully assess the health risk posed to the many fuel pump attendants, refinery and gasoline depots workers by prolonged exposure to inhalation gasoline, which generally saturate the ambient air of their workplaces.

In this study, we challenged male and female albino rats with gasoline vapour and monitored the endocrine disruptive effects as part of a comprehensive study of the health risks faced by refinery workers in Nigeria. The ultimate goal of the study included a definition of the potential impact of inhalation gasoline on reproductive and endocrine functions of the industry workers. Much information on the toxicological effects of gasoline vapour on humans in the country is relatively scanty. However, casual relationships have been variously made between gasoline inhalation and respiratory and central nervous system depression and death (Steffe *et al*, 1960, renal cancer, liver toxicity and risk of developing leukemia and myeloma (Enterline, 1993, Orzechowski *et al*, 1995) in many other countries.

Materials and Methods

Animals:

Inbred male and female albino rats (wistar) aged between 15-20 weeks weighing between 110g-130g were conditioned for this study in our laboratory. The animals were exposed to 12:12 hours of light/dark periods and allowed free access to water and their accustomed diet of grower's mash (Guinea feeds, Nigeria). The animals were randomized into four groups (A-D) of male or female rats each as follows: Group A (female test group n = 10), Group B (male test group n = 10), Group C (female control group n = 5), and Group D (male control group n = 5). All the female rats were confirmed to be cycling normally and to be sexually mature. This study was approved by the Research Ethics Committee of the Nnamdi Azikiwe University Medical School. The animal care guidelines of European Centre for the Validation of Alternative Methods (ECVAM, 1993) were followed.

Gasoline Challenge:

The members of Groups A and B consisting of 10 females and 10 males were exposed to gasoline vapour for 1 hour daily for 21 consecutive days. Exposure was achieved by soaking 5ml of commercially procured gasoline in 20g of cotton wool, which were plastered on one end of the rate cages. The vapour was allowed to mix with the ambient air of the cages and the larger environment and about 1-2ml of the gasoline was vapourized in the ambient air of each rat cage for the one hour. The exposure modality simulated a general occupational oil depot environment in which gasoline and other derivatives saturated the ambient air to which unprotected oil workers in Nigeria, are exposed daily for hours.

Monitoring the Estrus Cycling

The estrus cycling of the female test and control groups was monitored beginning from the Day-1 of exposure. Estrus cycling was determined by the vaginal swab method. The vaginal smears were stained with Leishmann's stain and examined under a light microscope.

Hormonal Assay

Serum samples were taken from all the animals (Groups A – D) after the 21 – day challenge. All samples were stored at zero degrees centigrade and analyzed within 24 hours after collection. The levels of LH, FSH, Progesterone, Estradiol and Testosterone were determined in the female and male animals. The determination was made by the microwell enzyme-linked assay method using respective hormone's kit (Syntron Bioresearch Inc. USA).

Results

Effect of Gasoline Inhalation on Estrus Cycling.

The cycle of the female test animals (Group A) was categorized into estrus weeks (Week I to V) covering the 21 days of challenge. The results show that by the third estrus week, only 6 of the 10 individuals in the female test group were still cycling and by the IV and V weeks, all the female rats exposed to gasoline had persistent diestrous indicating arrest of estrus cycling by gasoline inhalation. The results are summarized in table 1.

Hormonal Assay

The results of the hormonal assay of the male and female animals are summarized in table II. The hormonal profile of the male test group show a significant ($P<0.05$) decrease of the serum levels of testosterone at a mean of 2.7± 0.2 ng/ml relative to a mean level of 4.4 ± 0.7ng/ml for the male control group. On the other hand, the levels of LH and FSH in the male test group were significantly higher than the levels of the male control group. Gasoline inhalation appears to significantly, cause a rise in the levels of LH and FSH in the male rats as shown in table 2.

The hormonal profiles in the female test group show significant ($P<0.05$) suppression in the levels of Progesterone and Estradiol (16± 2.0 vs. 31± 18 vs 610 ± 40 ng/ml) respectively. However, whereas the level of FSH in the female test group was higher relative to the control animals, the level of LH in the test group was significantly ($P>0.05$) lower than that of the control animals with means of 3.5 ± 0.5 vs. 6.5 ± 0.9 miu/ml (table 3).

Table 1: Effects of 21-day gasoline inhalation on the Oestrous cycling of albino rats (Wistar)

Cycling Week	Test (n = 10) No. cycling	Control (n = 5) No. cycling
I	10	5
II	10	5
III	6*	5
IV	0**	5
V	0**	5

**Reduction in no of animals undergoing estrus cycling*

***No animal in this group was cycling, cycle arrested at diestrus.*

Table 2: Hormone profile of female albino rats after a 21-day exposure to gasoline inhalation

Hormone	Test (n=10)	Control (n = 5)
Pregesterone (ng/ml)	16±2.0	31±3.5
Prolactin (ng/ml)	7±1.0	11±1.3
LH (miu/ml)	3.5±0.5	6.5±0.9
FSH (miu/ml)	8±1.0	6±0.8
Estradiol (miu/ml)	285±18	610±40

Values = mean ± SD.

Table 3: Hormonal profile of male albino rats after twenty one (21)-day gasoline challenge

Hormone	Test (n=10)	Control (n = 5)
Testosterone (ng/ml)	2.7±0.2	4.4±0.3
Prolactin (ng/ml)	8.0±6.0	7.0±0.5
LH (miu/ml)	13±1.0	7.0±0.5
FSH (miu/ml)	0.5±0.5	2.5±0.28
Corticosteroid (ng/ml)	2.5±0.16	3.8±0.2

Values = mean ± SD.

Discussions

The goal of this study is to determine the potential influence of inhalation gasoline on endocrine and reproductive structures and functions. We had used animal models as part of a preliminary risk-assesment of occupational exposure to fuel vapour in the fast expanding Nigerian oil industry. Our earlier report showed profound disruption of the histopathological organization of the reproductive organs of albino rats after a moderate fuel vapour exposure (Ugwoke et al, 2004). The histopathological analyses of the reproductive organs of uterus, fallopian tube and ovary showed mild disruption over this relatively short exposure period, however these organs showed only xanthogranumatous depositions while the testes showed mild spermatocytic arrest. In this study we report significant alterations in the levels of the reproductive hormones of the animals. The levels of estrogen, progesterone and testosterone in the female and male rats were significantly ($P>0.05$) suppressed (tables 2 and 3). This suppression was also indicated by the arrest of the estrous cycling of the female rats at diestrus (table 1).

Hydrocarbons found in fuels and solvents are common in the oil industry environment and the occupationally exposed persons continuously inhale these compounds. The effect of vapourized fuel on the endocrine system is only poorly understood. The pre-ovulatory LH level of female US Air force Personnel is reportedly lowered by exposure to vapourized fuel (Susan *et al*, 2002).

Our present report suggests a possible endocrine disruptive effect by uncertain compounds in fuel. The effects of fuel on the levels of these hormones in the rats show significant suppression of the LH levels of the female test animals. This low LH level may negatively affect other LH – dependent physiologic functions including ovulation. On the other hand, the elevation of Estradiol and Progesterone levels with a concomitant significant suppression of FSH in the female animals is consistent with a compensatory response arising from reduced negative feedback on the hypothalamus-pituitary levels by the lowered Estrogen and Progesterone levels. This suggests that the effect of the fuel was at the level of the gonads. Gonadal level influence is further suggested by the lowering of the serum testosterone levels of male rats challenged with gasoline vapour. The animals had a significantly ($P<0.05$) lower testosterone level relative to their control counterparts (table 2). These abnormal hormonal levels suggest a disruption of steroidogenesis function in rats exposed to gasoline vapour. Translation research will be required to determine the reproduction health risks faced by occupational exposure to gasoline. The future direction of this study would be to assess adverse outcome on human subjects occupationally exposed to fuel vapour. There are sex and species-specific differences in outcome of exposure to fuel. It remains uncertain what compound(s) in gasoline caused the observed effects. Further research would also be required to decipher what constituent(s) may be responsible for the observed effects.

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